

NEW FINDINGS REGARDING LIGHT INTENSITY AND ITS EFFECTS AS A ZEITGEBER IN THE SPRAGUE-DAWLEY RAT

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INTRODUCTION

Circadian rhythmicities are oscillations of physiological cycles designed to create temporal organization. Circadian rhythms ensure that physiological mechanisms are expressed in proper relationship to each other and the 24 hour day. Light is the main zeitgeber ("time giver") for biological clocks. The daily variations in light intensity from dawn to dusk, and seasonally due to the rotation of the earth, act upon organisms to give them photoperiodic information. This entrainment allows them to vary biologically to prepare for reproduction, hibernation, migration and the daily adaptations necessary for survival.¹ In most mammals, the suprachiasmatic nucleus of the anterior hypothalamus has been implicated as the central driving mechanism of circadian rhythmicity. The photic input from the retina, via the retino-hypothalamic tract, and modulation from the pineal gland help regulate the clock. In this study we investigated the effects of low light intensity on the circadian system of the Sprague-Dawley rat. A series of light intensity experiments were conducted to determine if a light level of 0.1 Lux will maintain entrained circadian rhythms of feeding, drinking, and locomotor activity.

The intensity of light to which an animal is exposed is one determinant of the length of the free-running period of a circadian clock in a constant environment. In 1960, Jürgen Aschoff showed that more intense light shortens the free-running period in diurnal organisms, but lengthens the period in nocturnal animals. Aschoff further concluded that under more intense light, the time an animal is active, compared with the time it is at rest, increases in diurnal animals, but decreases in nocturnal animals. Also, in diurnal species, the total amount of activity during a free-running

period increases with light intensity, while the reverse is true in nocturnal species.²

METHODS

Groups of six male Sprague-Dawley albino rats (initial weight 250 g; final weight 350 g) were obtained from Simonsen Laboratories. Rats were selected from the lowest shelves on the racks by the vendor in order to select animals with exposure to low light intensity, thus controlling for possible retinal damage. Typical lighting levels in many animal vivariums exceed 1000 Lux. At this level it is now known that retinal damage can occur in albino rats. It is also important to establish a light history on the animals, because in circadian rhythm research, prior exposure to light can elicit aftereffects lasting up to 100 days.¹

The rats were housed individually in Nalgene metabolism cages made of lexan and polycarbonate. These materials pass all wavelengths of light in the visible spectrum, and a portion of the ultraviolet spectrum. We used a full-spectrum fluorescent light source (Duro-Test Vita-Lite) simulating the spectral qualities of natural sunlight. The metabolism cages were housed in individual light tight cabinets. The fluorescent light sources were located centrally over each metabolism cage. Using a calibrated radiometer, light intensities were adjusted to the appropriate experimental levels and set no higher than each specific intensity at the eye level of each animal.

An initial baseline intensity of 10 Lux was tested because previous studies in laboratory rodents indicated entrainment at this level and above. An experiment was performed utilizing a light intensity of 5.0 Lux, a level determined by consensus at a NASA Lighting Requirements in Microgravity Workshop.³ Two additional experiments were performed utilizing 1.0 Lux and 0.1 Lux (a level less than that of full moonlight, approximately 0.4 Lux).

The following protocol was used for each experiment: The rats were acclimated for a period of 1 week to their new environment at each light intensity. The light cycle was adjusted for a 12 hour period of light, starting at 0700, and 12 hours of darkness beginning at 1900. This 12L:12D cycle at each intensity was imposed for 3 weeks. Immediately following the 12L:12D cycle, the rats were exposed to a 2 week period of constant light (LL). At the conclusion of the LL period, the 12L:12D light cycle was reinstated for 1 week to re-entrain the animals. Daily animal care was performed in the dark at time intervals outside the range

of circadian entrainment (18 hours before or 33 hours after last maintaining the rats). A final experiment was conducted using 40 Lux for the 12L:12D cycle, followed by an 18-day period of constant darkness (<0.01 Lux) and a return to the LD cycle.

The data format represented below is a raster plot. It is a record of activity over a 24 hour period which is double plotted so that the pattern of the circadian rhythm can easily be seen by the unaided eye. This raster plot visually depicts the difference between the entrained and free-running periods at an intensity of 0.1 lux

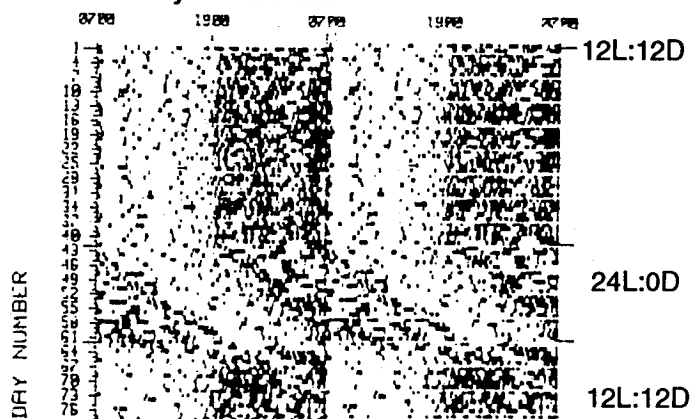


Figure 1

Table 1. Complex Demodulation Mean Period \pm S.E.

Lux	Activity	Drinking	Feeding
0.01	24.28 \pm 0.01	24.30 \pm 0.03	24.29 \pm 0.01
0.1	24.30 \pm 0.07	24.45 \pm 0.26	24.70 \pm 0.05
1	24.84 \pm 0.14	25.29 \pm 0.13	25.53 \pm 0.06
5	24.99 \pm 0.34	25.50 \pm 0.03	25.46 \pm 0.13
10	25.56 \pm 0.14	25.85 \pm 0.10	25.23 \pm 0.63

Period lengths were estimated from linear regression of acrophases (peak of harmonic fit to circadian rhythm) obtained from complex demodulation analyses [Table 1]. Cosinor analysis was utilized to obtain acrophase, mean amplitude, and significance level of the rhythms monitored.

RESULTS AND DISCUSSION

Visual inspection of the raster plots clearly indicated that circadian rhythmicity was maintained in all parameters monitored during LD. Results of cosinor analysis showed significant circadian rhythms ($\alpha=0.05$) were detected in all parameters during the control periods.

Furthermore, the results demonstrate that the free-running period length is directly proportional to the logarithm of light intensity [figure 2].

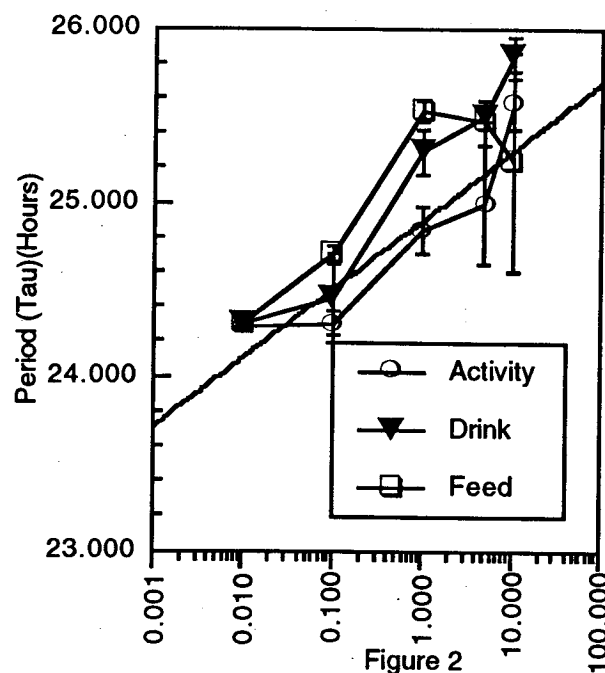


Figure 2
Light Level (lux)

This is consistent with Aschoff's Rule which states that the circadian frequency varies linearly with the logarithm of the intensity. With increasing light intensity, the circadian frequency of dark-active animals decreases (the period lengthens).

CONCLUSION

These experiments show that extremely low light levels (0.1 lux) can entrain the circadian system of the white laboratory rat. Therefore, the power requirement³ (5.0 lux) specified for Sprague-Dawley rats in microgravity is sufficient to maintain normal circadian rhythmicity.

1 Moore-Ede, M.C., F.M. Sulzman and C.A. Fuller, 1982. The Clocks That Time Us: The Circadian Timing System in Mammals. Harvard Univ. Press. [Boston].

2 Carpenter, Gail A., and Grossberg, S., 1984. A Neural Theory of Circadian Rhythms: Aschoff's Rule in diurnal and nocturnal mammals. Am. J. Physiol. 16:R1067-R1082

3 NASA Technical Memorandum 101077, 1988. editors: Holley, D.C., Winget, C.M., and Leon, H.A. Lighting Requirements in Microgravity-Rodents and Non-Human Primates. p.5-7.

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